STUDIES OF THE DAILY RHYTHMICITY OF THE FIDDLER CRAB, UCA. MODIFICATIONS BY PHOTOPERIOD[1]

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Since the first report of a persistent diurnal chromatophore rhythm in Uca by Abramowitz (1937), considerable work has been done in this laboratory as well as others in an attempt to elucidate the mechanisms controlling this periodic response. These studies, together with the reports of workers concerned with other manifestations of diurnal periodicity of function, have produced a literature which is extensively reviewed in Welsh (1938) and Webb (1950).

Light is the most obviously diurnally periodic feature of most environments and for this reason has been a popular experimental variable in studies of the control of diurnal rhythms. However, despite this fact, no work has been reported studying the effect of varying the extent of the daily photoperiod on the quantitative relations of a diurnally periodic response. The diurnal chromatophore rhythm of Uca can be readily observed and use of the Hogben-Slome scale for measuring chromatophore dispersion allows an accurate quantitative description of the characteristics of this rhythm. These characteristics make Uca a suitable experimental animal for such purposes. Thus the experiments to be reported were designed to contribute to an understanding of the mechanisms controlling diurnal periodicity by studying the character of the responses of this chromatophore rhythm to variations in the length of the daily light period to which the animals are exposed.

Material and Methods

All animals used in these experiments were male specimens of *Uca pugnax* collected in the vicinity of Woods Hole, Massachusetts. The animals were placed in white enameled pans in groups of twenty and subjected either to illumination at an intensity of 100 foot candles or to darkness, depending on the protocol which the group concerned was to follow. This was done not later than two hours after collection. The time of collection was between 10 A.M. and 4 P.M., this time being chosen to avoid the dark period of as many of the experimental groups as possible in order to provide constant experimental treatment.

Two sets of experiments are to be reported which differ to some extent in protocol. All succeeding references to light refer to light at an intensity of 100 foot candles furnished by the laboratory fluorescent lights. Groups were subjected to indicated periods of darkness by placing them in an adjacent darkroom. In both sets of experiments the condition of the black chromatophores in each experimental group was determined at 1, 4, 7, and 10 A.M., and 1, 4, 7, and 10 P.M. by

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recording the extent of dispersion for each animal in terms of the Hogben-Slome scale.

The first set of experiments was begun on July 26, 1950 and terminated on August 9, 1950. The following groups were maintained and the black chromatophore activity of each ascertained in the manner described above.

1) A control group maintained in constant darkness from 4 P.M. July 26 until the experiments were concluded.
2) A group exposed to light from 7 A.M. to 1 A.M. and darkness from 1 A.M. to 7 A.M. daily (18-hour photoperiod).
3) Exposed to light from 1 A.M. to 7 P.M. (18-hour photoperiod).
4) Exposed to light from 4 A.M. to 10 P.M. (18-hour photoperiod).
5) Exposed to light from 7 A.M. to 1 P.M. (6-hour photoperiod).
6) Exposed to light from 1 P.M. to 7 P.M. (6-hour photoperiod).
7) Exposed to light from 10 A.M. to 4 P.M. (6-hour photoperiod).

In each case not specified above, the animals concerned spent the remainder of each 24 hour period in darkness and were accorded the same treatment daily for the duration of this portion of the experiments. On July 31 groups 2 through 7 above were placed in darkness after exposure to light as outlined above and so maintained until the experiments were terminated on August 9. There are two exceptions to this statement; one-half the animals remaining in groups 3 and 4 were placed in darkness at 1 P.M. and 4 P.M., respectively, on July 31. It was found that this treatment altered neither the amplitude of the rhythm nor the magnitude of the dispersed phase and produced only a transient effect on the time of concentration. After this had been ascertained, these animals were discarded and are not included in the calculation of the magnitude of the dispersed phase for the groups which will be discussed subsequently. Thus, at the end of this set of experiments, groups 3 and 4 were smaller than the others, containing respectively seven and five animals. In no other case did less than 10 of the original 20 animals in each group survive the experimental period.

The second set of experiments was begun on August 14, 1950 and terminated on August 24, 1950. The following groups were maintained and black chromatophore activity ascertained in each case as before.

1) A control group maintained in constant darkness as before for the duration of the experiments.
2) A second control group was maintained in conditions as closely approximate to normal with respect to light periodicity as feasible. The animals were exposed to daylight in a wooden collecting bucket. No attempt was made to control or measure intensity but the animals were shielded from direct sunlight to avoid lethal temperatures. Sea water in the bucket was renewed daily and the animals were shielded from rain when necessary.
3) Exposed to light from 4 A.M. to 10 P.M. daily (18-hour photoperiod).
4) Exposed to light from 10 A.M. to 4 P.M. daily (6-hour photoperiod).

On August 18, groups 3 and 4 were placed in darkness after their respective exposures to light (in the case of group 2 at 1 A.M. on August 20) and so maintained until the experiments were terminated. In no case did less than 9 of the original 20 animals in each group survive the experimental period.

The diurnal rhythm of the black chromatophores persisted in all cases. For the purposes of analysis of the data this rhythm has been treated as a daily excursion into the dispersed phase and direct measurements of the course of each 24 hour cycle transformed into a parameter which is proportional to the area swept out by the dispersed phase. The formula used for this calculation is

$$A = \frac{\sum_{i=1}^{n} (x_i - 1)}{8}$$

"i" refers to the time of the reading concerned. "i = 1" was arbitrarily taken as the initial point of minimum dispersion in the cycle under consideration.

"x_i" is the average extent of dispersion of the black chromatophores of the group concerned as measured in terms of the Hogben-Slome scale at the time "i".

"n" is the number of the reading which indicates the second point of minimum dispersion and the conclusion of the cycle considered. The magnitude of "n" varied between 8 and 10. Reading "n" for one cycle is then reading "1" for the cycle immediately succeeding.

This formula essentially gives an expression for the average dispersion of the black chromatophores in the course of one cycle, on the assumption that each cycle occurs in 24 hours. This assumption is thoroughly justified on the basis of the observational data.

Results

The values for the average dispersion of the black chromatophores for each group calculated, using the formula explained above, are arranged in tabular form

Table I

Daily magnitude of the dispersed phase in each group of the first series of experiments. All groups in constant darkness after the fifth day

Group..... Day	1	2	3	4	5	6	7
1	2.10	2.33	2.45	2.39	1.63	1.81	1.94
2	2.36	2.45	2.66	2.66	1.76	2.00	2.11
3	2.71	2.46	2.65	2.64	2.04	1.81	2.09
4	2.50	2.64	2.61	2.64	2.16	2.07	2.21
5	2.32	2.69	2.76	2.81	2.11	1.89	2.14
6	2.56	2.57	2.23	2.56	2.26	1.99	2.11
7	2.53	2.23	2.29	2.28	2.16	1.93	2.13
8	2.51	2.25	2.29	2.35	2.28	2.36	2.09
9	2.89	2.49	2.32	2.50	2.16	1.99	2.14
10	2.61	2.45	2.41	2.06	2.20	2.23	2.10
11	2.46	2.29	2.20	2.05	1.97	1.76	1.98
12	2.53	2.39	2.21	2.18	2.18	2.02	1.96
13	2.58	2.50	2.39	2.13	2.02	2.06	1.85
Av. (6-13)	2.58	2.40	2.30	2.26	2.16	2.04	2.04
SD	±.08	.11	.06	.16	.08	.13	.09
Aggregate average		2.32 ± .14			2.08 ± .11		

in Tables I and II. Inspection of the values for the first five days of the first series of experiments as presented in Table I is sufficient to validate the statement that for this period the responses of groups 2, 3, and 4 are similar and differ significantly from the responses of groups 5, 6, and 7. Further, the responses of groups 5, 6, and 7 are similar. The antepenultimate row of Table I lists the average value of the magnitude of the dispersed phase in cycles 6 through 13 of the groups concerned and the penultimate row gives the standard deviation for this value. These cycles were chosen as representative of what may be called the resting level of the rhythm in constant darkness. It is seen that these average values fall naturally into three sets comprised of group 1, groups 2, 3, 4, and groups 5, 6, 7. The last row of Table I lists the averages of cycles 6 through 13 for groups 2, 3, 4, and groups 5, 6, 7 considered as aggregates, together with the standard deviation of these values. This constitutes evidence for the conclusion that the magnitude of the dispersed phase in the rhythm, both during the periodic light exposure and in constant dark-

TABLE II

Daily magnitude of the dispersed phase in the second series of experiments. All groups in constant darkness after the fifth day except 2 which was placed in darkness on the sixth day

Group / Day	1	2	3	4
1	1.35	2.30	2.23	1.59
2	1.38	2.11	2.44	1.79
3	1.59	2.01	2.54	1.69
4	1.80	2.13	2.48	1.80
5	1.95	2.06	2.65	1.84
6	2.18	2.20	2.19	1.84
7	2.11	1.54	2.33	1.94
8	2.13	1.41	2.32	1.79
9	2.18	1.59	2.16	1.73
10	1.99	1.80	2.09	1.67
Av. (6–10)	2.12		2.22	1.79
Av. (1–6)		2.13		
SD	±.05	.08	.09	.08

ness afterwards, is influenced by the length of daily exposure to light but is uninfluenced within the limits of the experimental procedure by the time of day at which the animals are exposed.

Table III lists the deviations of the minimum dispersion point at the start of each cycle for each group from an arbitrary zero point of 1 A.M. Two conclusions are supported by these tabulated data. On July 26, 1950, time of sunrise was 0530 Eastern Daylight Saving time and time of sunset was 2008. This places the middle of the naturally-occurring dark period at −0.18 in terms of the units used in Table III. Similar considerations place the middle of the naturally-occurring dark period on August 14, 1950 at −0.23. Given these facts, reference to the average values in the last row of Table III for series I, group 1 and series II, groups 1 and 2 allows the conclusion that the point of minimum dispersion does not normally coincide with the middle of the natural dark period nor does it bear any simple fixed relation to it. Evidence is provided for a second conclusion by inspection of the average deviations

for series I, groups 2 and 3. Both groups were exposed to light for 18 hours per day but the middle of the experimental dark period was at 4 A.M. and 10 P.M. respectively. The respective increments with respect to the middle of the normally-occurring dark period on July 26 are 3.18 and −2.82. The measured increments are 2.96 and −1.86. That the induced shifts are permanent is attested by the fact that the averages of points 6 through 14 (*i.e.*, after the animals were in constant darkness) are 2.67 and −2.33 in the two cases, manifesting no tendency to drift back to the control value represented by group 1 in this series. The value for series II, group 3 is further evidence for the existence of a persistent shift. It should be pointed out that since measurements were made at three-hour intervals, the error for each of the tabulated values is high, of the order of an hour and a half. However, the probable error of the average of a number of such values is considerably smaller and does not vitiate the conclusions drawn.

TABLE III

Daily deviations of the point of minimum dispersion from 1 A.M. as an arbitrary zero point. Time in hours

	Series I							Series II			
Group... Day	1	2	3	4	5	6	7	1	2	3	4
1	0	3.0	0	0	0	0	0	0	−3.0	1.5	−1.5
2	0	3.0	−1.5	0	1.5	1.5	1.5	1.5	0	0	−1.5
3	1.5	3.0	−1.5	1.5	3.0	3.0	3.0	−1.5	0	0	−1.5
4	0	3.0	−1.5	0	−1.5	0	0	0	−3.0	0	−1.5
5	3.0	3.0	−3.0	0	−1.5	−3.0	0	−1.5	−1.5	0	−1.5
6	0	3.0	−1.5	0	−1.5	0	−1.5	−1.5	−1.5	0	−3.0
7	3.0	3.0	−3.0	−3.0	−3.0	3.0	0	−1.5	−1.5	0	0
8	1.5	1.5	−3.0	−3.0	0	0	0	−3.0	−1.5	0	−1.5
9	0	1.5	−1.5	−3.0	0	0	0	−1.5	0	0	−1.5
10	1.5	3.0	−3.0	−1.5	0	0	0	−3.0	−1.5	0	−3.0
11	1.5	3.0	−3.0	−3.0	1.5	3.0	0				
12	0	3.0	−3.0	−1.5	−1.5	−3.0	−1.5				
13	0	3.0	−3.0	−1.5	0	0	0				
14	0	3.0	0	0	−1.5	0	0				
Av.	.86	2.78	−2.04	−1.07	−.32	.32	.11	−1.20	−1.35	.15	−1.65

Figure 1 displays graphically the variation in the magnitude of the periodic dispersion of the black pigment in the first series of experiments. It has been previously argued that the time of day at which the animals were exposed to light was immaterial in a consideration of induced shifts in this magnitude, so that the data from groups 2, 3, and 4 have been averaged to give the points representing the response of animals exposed to 18 hours of light daily and the data from groups 5, 6, and 7 contribute in the same way to the curve for animals exposed to 6 hours of light daily. Figure 2 presents the same information for groups accorded similar treatment in the second series of experiments and in addition records the variations in dispersion for the additional control group maintained under normal light conditions.

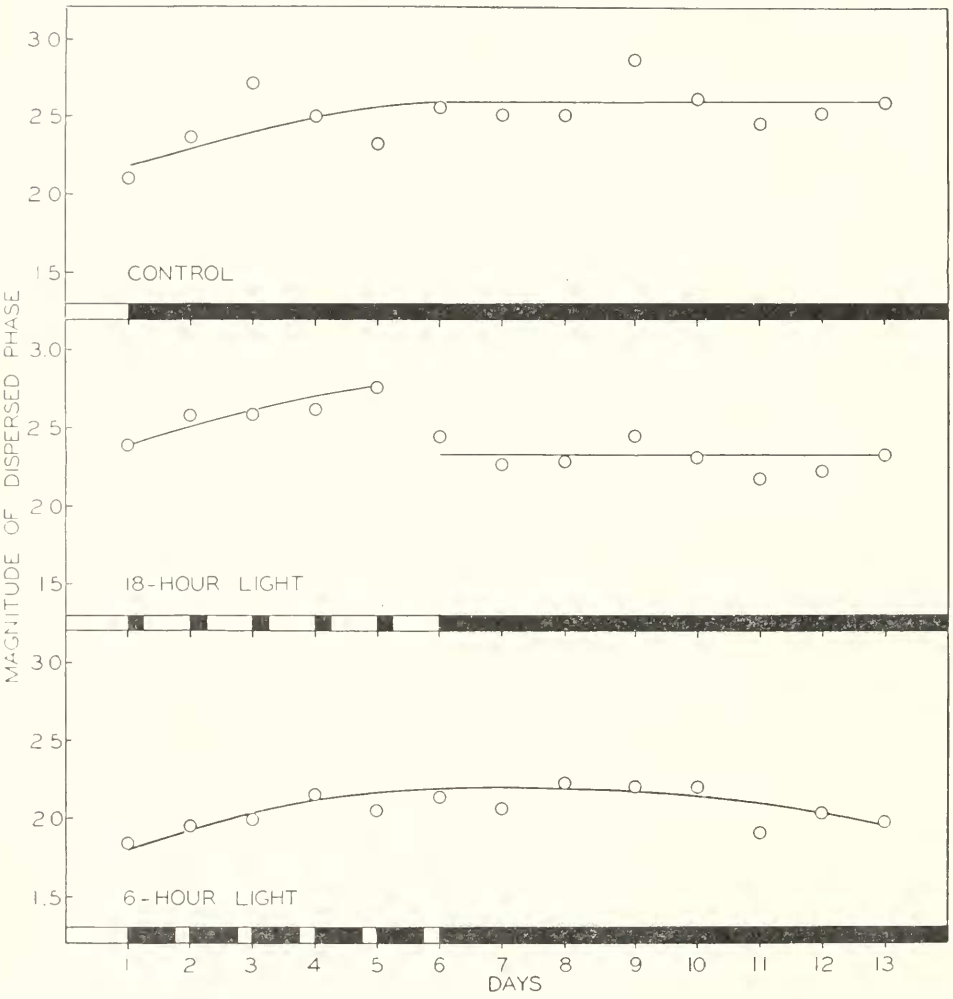

FIGURE 1. The responses of the magnitude of the dispersed phase of the chromatophore rhythm in the three groups of the first series of experiments. The white and black blocks under each curve are a diagrammatic representation of the relative lengths of each 24 hour period spent in light and darkness respectively.

There are several features of these graphs which are of interest. It will perhaps be simplest to discuss the responses of the groups receiving similar treatment in the two sets of experiments.

One of the most striking features of the control values in both figures is their smooth increase over a period of several days from an initial relatively low magnitude to attain finally a resting level. This is particularly pronounced in series II (Fig. 2) where the effect manifested by the controls in darkness is repeated almost quantitatively by the controls maintained in normal daylight when placed in constant darkness after the sixth day. This portion of the response is less evident in series I

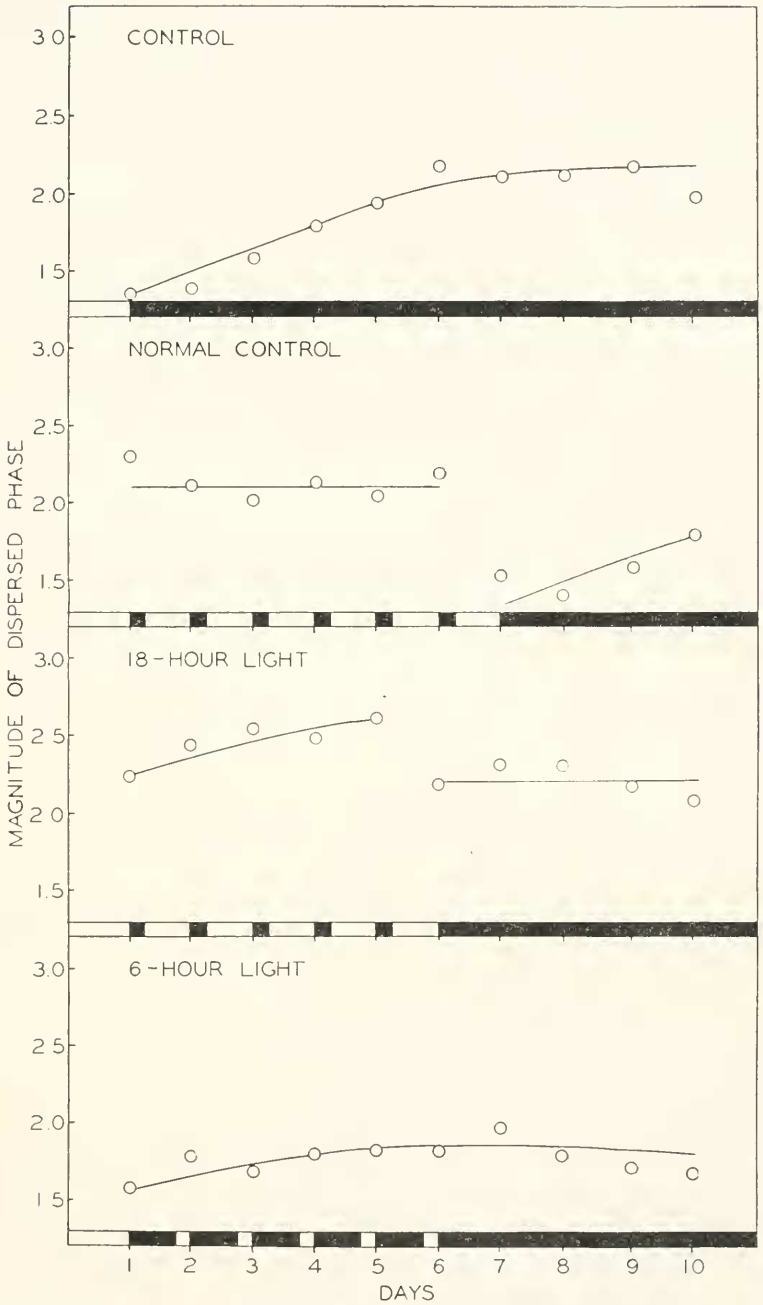

FIGURE 2. The responses of the magnitude of the dispersed phase of the chromatophore rhythm in the four groups of the second series of experiments. The white and black blocks beneath each curve have the same significance as in Figure 1.

although it can be discerned. It is of even greater interest in that no similar depression seems to intervene between the response while receiving a daily light ration and the subsequent resting level in darkness in either of the 18-hour or the 6-hour groups. Another point of interest is the correspondence between the resting level of the control group kept in darkness and the level maintained by the controls kept in normal daylight conditions. The average values and standard deviations for the two levels are 2.12 ± 0.05 and 2.13 ± 0.08 respectively. In the light of the character of these experiments, it would be of the greatest interest to be able to state the day-length to which the animals collected were exposed under natural conditions. The times of sunrise and sunset for July 26 and August 14 have been reported above. If these values be taken as the initial and final points of the light period, the day-length in the two cases is 14 hours 38 minutes and 13 hours 58 minutes, respectively. However, if the time for the beginning of morning twilight and end of evening twilight, as listed in American Ephemerix and Nautical Almanac for 1950, be taken as initial and final points, the day-lengths on the two days become 18 hours 39 minutes and 17 hours 28 minutes.

Animals exposed to 18 hours of light per day (series I, groups 2, 3, 4, and series II, group 3) display an increasing dispersion of the black chromatophores. This increase is apparently not immediately induced but represents a response extending over a period of several days. The initial value in both series is near the resting level of the control groups so that we may conclude that this cumulative increase operates on an initially normal value (see preceding paragraph) producing a characteristic smooth rise. As previously mentioned, in neither of the sets of experiments does a depression intervene between this phase of the response and the assumption of a resting level in darkness. It is further of interest that the levels assumed in the two cases are quite similar despite large discrepancies in the control and 6-hour experimental levels.

Animals exposed to 6 hours of light per day (series I, groups 5, 6, 7 and series II, group 4) manifest an initial depression (below resting level) and then rise steadily in the course of three or four days to assume a level which remains unchanged with the advent of continuous darkness. This level is significantly below the control level in both cases but appears to differ by about 0.2 units in the two sets of experiments.

Discussion

If we first consider the problem of the control of the magnitude of the periodic dispersion of the black chromatophores, several lines of reasoning indicate that two more or less independent controlling centers must be postulated. Let us first examine the response of animals exposed to 18 hours of light daily. The initial phase of the response is a modification of the rhythm which has as its starting point the normal magnitude. The cumulative character of this phase of the response indicates that it is not to be explained as a direct response, where "direct" is used to refer to a response not mediated by a rhythmical center whose activity is influenced by one or more types of environmental stimuli. The fact that the level subsequently assumed by the animals receiving this treatment in series I is significantly below the control level, although the values attained in the initial phase of the response are significantly above this level, indicates that the center whose

activity was cumulatively modified to produce the initial increment is not responsible for the level at which the rhythm stabilizes in continuous darkness.

An examination of the behavior of the control groups furnishes further support for the hypothesis of a dual control of chromatophore rhythmicity. The period of depression or inhibition evident in the response of all groups on being placed in constant darkness from normal conditions is smoothly released, indicating again a cumulative modification of the activity of a controlling center. That this is not the controlling center responsible for the resting level assumed in darkness is indicated by the significant difference between the resting level of the control groups in the two sets of experiments.

Let us consider the characteristics of the two centers thus supported and attempt to describe insofar as possible the stimuli by which the activity of each is modified and the relations between the two centers. We have then two centers, A and B, which are concerned with the control of the diurnal periodicity of the black chromatophores of Uca. If the following characteristics are postulated, an explanation of the reported results can be given.

1) Center A manifests a persistent 24 hour rhythmicity in the secretion of a black-dispersing principle and the extent of this secretory activity is modified only by changes in the length of the daily photoperiod to which the animal is exposed. In addition to a direct effect on the stage of black chromatophore dispersion, this secretion, or another concomitant activity of center A, exerts a controlling influence on the rhythmical activity of center B. Total darkness or a daily photoperiod below some threshold length (no experimental evidence is available to permit a choice between these alternatives) is not effective as a stimulus in changing the activity of center A.
2) Center B also manifests a 24 hour rhythmicity but a more labile one than center A, and less persistent. Center B gradually becomes completely synchronized with center A when environmental stimuli which normally influence center B are removed. Center B has as mediating secretions both a black-dispersing and a black-concentrating substance. It responds to darkness by secretion of the black-concentrating substance and to light by secretion of the black-dispersing substance at its disposal. More precisely, there is an intensity i such that at or below this intensity a black-concentrating principle is secreted, this response being graded, and there is another intensity j, which may or may not be identical with i, such that at or above this intensity a black-dispersing principle is secreted, this response also being graded.

With two centers having these characteristics, it is possible to give a coherent explanation of the results of the two series of experiments. Control groups, it will be remembered, responded to being placed in total darkness by an initial depression in magnitude with a gradual return to an original resting level. Since there were no stimuli which could effect a change in the activity of center A, and since centers A and B would normally be acting synergistically, it is reasonable that the resting level in darkness should equal the level maintained in normal daylight. Further, since center B would respond to darkness with an increased secretion of a concentrating principle, a depression of the rhythm magnitude is to be expected until A regains complete control of the periodic activity of B.

When animals are placed in 18 hours of light at an intensity of 100 foot candles the situation is somewhat complicated. Under normal conditions, the animal would be subjected to periods of low intensity at the beginning and at the end of the photoperiod, from which it follows that center B would be activated to produce a dispersing substance during only a portion of the normal day-length exposure. However, under experimental conditions the animals were exposed to an intensity of 100 foot candles throughout the photoperiod. Thus, B was activated to produce a dispersing substance at a time when it had not been so activated under natural conditions. Such activation modified not only the immediate response of B but the character of its rhythmical activity so that as the stimulus was repeated on successive days, the reaction of center B to light became more and more effective in producing chromatophore dispersion. Thus, we observed a cumulative increase in magnitude as the initial response of these groups. Although center B continued its increased activity in the production of dispersing substance after the animals were placed in continuous darkness, this in itself also operated as a stimulus to the production of a black-concentrating substance. Both of these effects would be transient as center A gradually established control of the activity of B, and since they are antagonistic, only one of them could be expected to be observed and it should be less pronounced than it would be if operating alone. In fact, neither trend is observable. On the basis of this hypothesis, since the natural photoperiod on July 26 was greater than 18 hours (18 hours 39 minutes), center A should stabilize the rhythm at a level somewhat below the control level. The natural day-length on August 14 being 17 hours 28 minutes, the resting level in 18-hour animals should exceed that for the controls. Both of these expectations are confirmed. It should be pointed out in this connection that there is no reason to expect the activity of center A to be linearly related to the length of the photoperiod, so that it suffices to show one effect is greater than another, provided the stimulating day-length for the first is longer without regard to quantitative correspondence of increments. Finally, it is reasonable that the resting level attained by the 18-hour animals in darkness should be the same despite discrepancies in control level if center A is responsible for the control of this response.

In the response of animals exposed to 6 hours of light daily, center B is responsible for the initial drop in magnitude by its secretion of concentrating substance in response to the increased darkness and this effect is later supported by the induced shift in activity of center A. However, since center A responds slowly to changes in the length of the light period, the total effect will be a slow rise mimicking that shown by control groups when placed in darkness. For the same reasons which were adduced in the discussion of the reaction of the 18-hour animals, either an increase or a decrease in magnitude could be produced when the animals were placed in continuous darkness and neither was observed (the decrease observed in both sets in the course of several days is probably too small to be significant). On the basis of this hypothesis it is reasonable that the levels in the two cases were not the same since, by hypothesis, center A responds slowly to a change in light period. This argument does not apply to the case of the 18-hour animals since the experimental value in that case was quite close in both sets of experiments to the natural photoperiod.

Centers A and B discussed in the preceding hypothesis are in no way incompatible with centers 1 and 2, respectively, of Brown and Webb (1949), which were postulated

to account for other responses of the rhythmic mechanism of Uca; in fact, they even exhibit the same intrinsic characteristics. They are, therefore, assumed to be the same and this work is considered to provide striking support for the earlier hypothesis and to describe further properties of these two postulated centers.

Figure 3 is a diagrammatic representation of the mechanism which has been hypothesized to account for the responses of the daily rhythmicity observed during these experiments. In conformity with the earlier work, centers A and B are here indicated as 1 and 2.

With regard to the elements involved in this hypothesis, there is no reason to be concerned with the identity or non-identity of the dispersing principles whose liberation is controlled by A and B. The hypothesis requires only that there be two controlling centers, each of which has at its disposal a dispersing substance. Although it is not necessary that these two centers be physically discrete, it is possible that they are. Thus, it is of interest that both the sinus gland and the central nervous system in Uca elaborate black-dispersing substances (Sandeen,

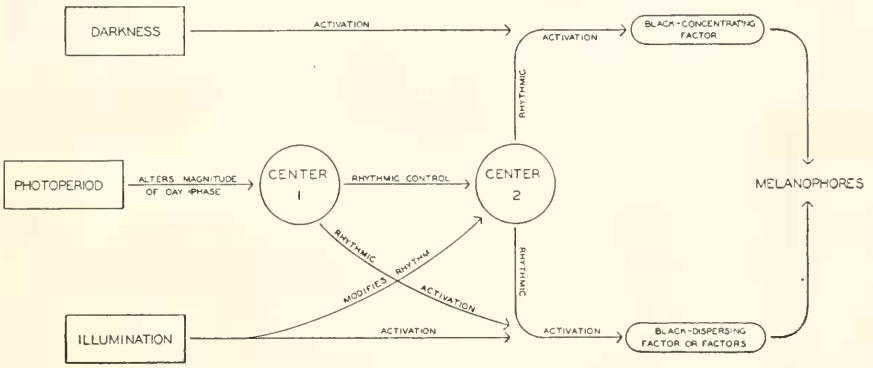

FIGURE 3. A diagrammatic representation of the postulated control mechanism of the black chromatophore rhythm of Uca.

1950). There is no direct evidence for the existence of a black-concentrating principle but other work done in this laboratory (Brown and Hines, in press) provides indirect support for its postulation.

The complexity of this controlling mechanism leads inevitably to speculation concerning the reasons for such elaborate systems. The idea has often recurred in the invertebrate endocrine literature that chromatophorotropins are simultaneously concerned with more basic functions. If, purely speculatively, this reasoning is followed, one may ask what functions would be thus controlled by the photoperiod. Panouse (1943, 1944, 1946), Brown and Jones (1949), and Stephens (1951) have demonstrated clearly the endocrine nature of the control of ovarian maturation, and the last investigator has shown a role of photoperiod in the decapod sex cycle. We have here a function which is presumably dependent on responses to change in day-length but which also requires that the responding system be uninfluenced by large short term variations in photoperiod. On the other hand, Edwards (1950) and others have pointed to the significance of endocrines in the diurnal activity rhythm

of the decapods. This, in contrast, requires an elastic response system capable of following short term variations. It is obvious that two such functions, if combined, produce a total chromatophorotropin system similar to the one described. Center A in our hypothesis represents an inelastic response mechanism whose characteristics are such as to make it suitable for the control of the sex cycle. Center B possesses the necessary lability for control of a function such as locomotor activity rhythm which must be warped to the exigencies of short-term environmental variations. Although there is some scattered evidence that some chromatophorotropins have other systemic effects in the decapods, such evidence can afford only general support for the possibility of this kind of interrelation which must remain speculation until further experimental evidence is accumulated. On the other hand, it is quite possible that these same two centers will be shown to exert their influences directly upon other nervous and endocrine mechanisms than those involved in color changes.

Table III provides evidence that permanent shifts in the relation of the phases of the diurnal rhythm to solar time, of the order of two to three hours in either direction from an arbitrary zero point, can be induced by suitable variation in the time of illumination of the animals. The data seem insufficient to warrant a more extended analysis or to validate less general conclusions, but merely indicate the desirability of a more refined approach to the problem of the control of the temporal relations of the diurnal chromatophore rhythm.

Summary

1. It has been shown that if the diurnal rhythm of the black chromatophores of Uca is treated as a periodic excursion into the dispersed phase, the magnitude of this excursion is significantly influenced by the length of the daily photoperiod to which the animals are subjected.

2. That persistent alterations are induced by different photoperiods is clearly seen in the levels at which the rhythm stabilizes after the animals are placed in constant darkness subsequent to experimental treatments with light.

3. The time of an 18-hour period of illumination between 1 A.M. and 1 A.M. appears not to influence the induced magnitude of the rhythm. The time of a 6-hour period of illumination between 7 A.M. and 7 P.M. is similarly immaterial. However, it has been shown that variations in the time of day within these limits of the period of illumination can produce corresponding and persistent shifts in the relation of the phases of the rhythm to solar time. These alterations may be in either direction from an arbitrary zero point.

4. In addition to the induction of persistent modifications of the rhythm by photoperiod, the character of the rhythm has been shown to be influenced also by illumination and by darkness. These latter effects are transient when the animals are no longer exposed to alternating periods of light and darkness.

5. An analysis of the data clearly indicates the operation of two centers of rhythmicity, such that the activity of the first is influenced by the length of the photoperiod to which the animal is exposed while the activity of the second is influenced by the duration and intensity of light to which the animal is exposed daily, by the duration of the daily dark period, and by the activity of the first center.

6. An adaptive significance for this duality of the control of the rhythm is suggested.

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